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Short Communication

Relationship between numbers of birch pollen and different particle sizes of the pollen antigens (Bet v) in the air in Stockholm, Sweden

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Abstract

Micronic antigens penetrate to the lower respiratory tract and cause serious lung problems. The existence of micronic antigens in pollinosis-caused pollen has been reported. We examined the appearance and the relationship of micronic particles of birch pollen antigens and birch pollen grains. Airborne particles were collected with a tandem filter system sampler composed of a 5 μm pore filter and a 0.3 μm pore Millipore filter in springtime at the Stockholm University campus in Stockholm. A fairly good correlation was observed between numbers of birch pollen and the amount of birch pollen antigens above 5 μm in diameter. In contrast, a good correlation was not observed between the amount of birch pollen antigens collected in 5 μm pore filters and the amount of the antigens passing through 5 μm pore filters and collected on Millipore filters. The appearance of a maximum value of birch pollen antigens collected on Millipore filters seemed to be delayed a few days after a maximum values had been obtained for birch pollen on a Burkard sampler or birch pollen antigens on 5 μm pore filters. Almost all birch pollen antigens were found during the birch pollen season and recognizable amounts of birch pollen antigens were not detectable after the birch pollen season. Most of the birch pollen antigens collected on 5 μm pore filters originated from birch pollen grains. In contrast, the antigens passing through 5 μm pore filters and collected on Millipore filters did not originate from pollen grains themselves, but were micronic particles, and appeared a few days after maximum values of birch pollen had been obtained.

Key words: airborne antigen, antigen, Bet v, Birch pollen, micronic particle, pollen.

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Introduction

The existence of micronic antigens in many pollinosis-caused pollens, such as ragweed (Ambrosia),[1] birch (*Betula*),[2] grass (Gramineae)[3,4] and Japanese cedar (*Cryptomeria japonica*)[5] pollen, has been reported. Micronic antigens penetrate to the lower respiratory tract and cause serious lung problems, like pollen asthma and bronchial disorders. We have previously reported that micronic particles carrying Cry j 1, one of the major pollen antigens of Japanese cedar pollen, appeared after dispersion of a large number of pollen grains.[5] There have been only a few reports[6–8] concerning pollen asthma caused by Japanese cedar pollen, despite considerable pollen dispersion; in fact, more than 15% of Japanese are considered to suffer pollinosis due to Japanese cedar pollen. In contrast, there have been several reports related to micronic antigens of birch.[9–12] The number of pollen asthma-like patients increased after dispersion of a large amount of birch pollen. These observations led us to compare the amount of micronic birch pollen antigens in Stockholm,

Sweden, with our previous results obtained with Cry j 1 in Yamagata, Japan, using the same sampling method and the same antigen-measuring technology.

METHODS

Sampling procedure

Airborne pollen antigens were sampled with a tandem filter system sampler composed of a 5 μm pore capillary filter (Nuclepore filter; Nuclepore, Pleasanton CA, USA) and a 0.3 μm pore Millipore filter with a diameter of 47 mm. The sampler was placed on the rooftop of a building on the Stockholm University campus in Stockholm. Samples were taken from early April to the middle of July 1998. The filters were replaced regularly every 72–96 h at about 08.00 h. Each filter was put in a plastic dish and stored at –20°C until use.

Pollen sampling was performed with a Burkard 7 day recording spore and pollen trap (Burkard, Rickmansworth, UK). The trap was placed on the rooftop adjacent to the tandem filter sampler for airborne pollen antigen detection. The Melinex tape was coated with white vaseline and changed weekly. Methods for counting airborne pollen have been described previously.[13]

Meteorological data (temperature, relative humidity) were gathered from the Bromma Station in Stockholm, located 2 km north-west from the University campus.

Bet v extraction and detection

Bet v was extracted with 1 mL of 0.125 mol/L NH_4HCO_3–0.01% bovine serum albumin (BSA) solution at 4°C for 24 h.

The amount of Bet v in the extracts was measured by fluorometic sandwich ELISA.[14] Briefly, microplate wells were coated with rabbit anti-Bet v IgG. Reference Bet v extracts were also incubated in the wells. The wells were incubated with biotinylated anti-Bet v rabbit IgG then with β-D-galactosidase-conjugated streptavidin, and finally with 0.1 mL of 0.1 nmol/L 4-methyl umbelliferyl-β-D-galactoside. After incubation for 2 h at 37°C, the fluorescence intensity was measured with a microplate fluorometric reader (Fluoroskan; Flow-Laboratories, McLean, MD, USA). The results are expressed as ng per filter. The sensitivity of this ELISA was 0.1 ng/mL Bet v. Rabbit anti-Bet v IgG was a gift from ALK Laboratories (Hörsholm, Denmark).

RESULTS

Alder (*Alnus* spp.) pollen appeared prior to birch (*Betula* spp.) pollen (Table 1). The most common pollen genus observed at the time of our investigation period was birch. A fairly good correlation was observed between the amount of birch pollen and the amount of birch pollen antigens on 5 μm pore filters. In contrast, a good correlation was not observed between the amount of birch pollen antigens on 5 μm pore filters and those on Millipore filters. The appearance of a maximum value of micronic birch pollen antigens collected on Millipore filters was delayed a few days after a maximum value of birch pollen had been obtained (Fig. 1). Considerable dispersion of pollen continued for approximately 2 weeks. A significant amount of birch pollen antigens was not found after the birch pollen season. Sudden rises in temperature initiated a large dispersion of birch pollen and a considerable amount of birch pollen antigens and micronic birch pollen antigens was observed several days after birch pollen dispersion. However, an intercorrelation was not observed between the appearance of birch pollen, the amount of birch pollen antigens and some of the meteorological factors, as shown in Table 2.

Table 1 Basic values relevant to pollens and antigens

	Date of maximum pollen count	Maximum pollen count or maximum antigen amount	Total pollen count or total antigen amount
Alder pollen	24–27 April	144 grains/m^3	419 grains/m^3
Birch pollen	8–11 May	6968 grains/m^3	17 453 grains/m^3
Bet v antigens above 5 μm	8–11 May	7600 ng	20 118 ng
Bet v antigens below 5 μm	11–12 May	43 ng	244 ng

Sampling was performed between 6 April and 14 July.

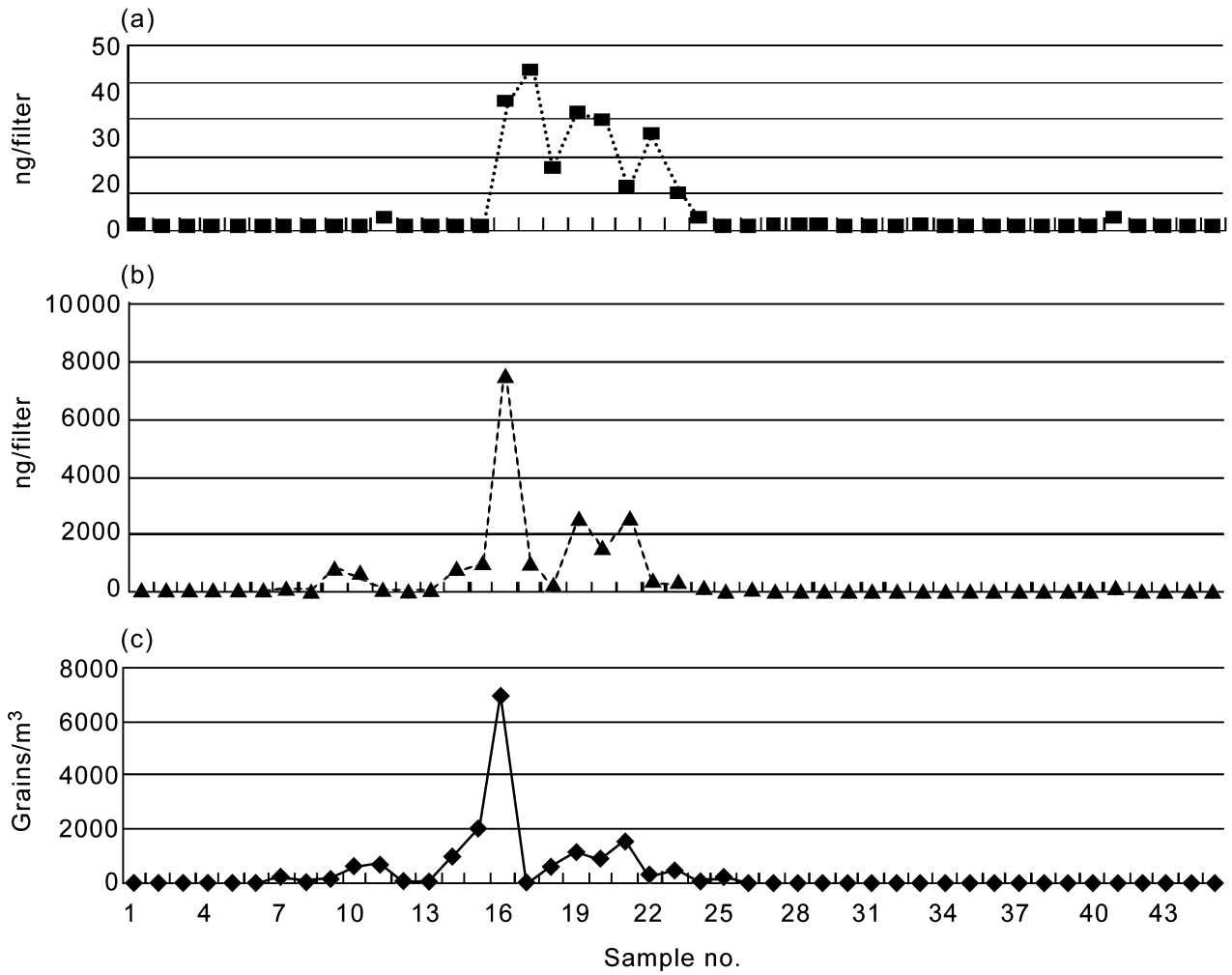

Fig. 1 Relationship between airborne Bet v and the pollen count. Bet v on (a) Millipore and (b) 5 μm Nucleopore filters was quantified with sandwich ELISA. (c) Birch pollen was counted morhpologically with a Burkard sampler, which was placed adjacent to the tandem filter system.

Table 2 Correlation coefficients between Bet v antigens below 5 μm, Bet v antigens above 5 μm, birch pollen and meteorological conditions

	Bet v antigens		Birch pollen
	< 5 μm	≥ 5 μm	
Bet v antigens ≥ 5 μm	0.646		
Birch pollen	0.501	0.953	
Temperature			
Maximum	0.298	0.290	0.234
Mean	0.173	0.203	0.168
Minimum	−0.018	0.070	0.085
Humidity	−0.425	−0.323	−0.257

Discussion

Values of micronic antigens obtained from our tandem sampling system will underestimate the actual values that exist in the air because some micronic antigens will adhere to the 5 μm pore Nuclepore filter despite the fact that they are smaller than the pore size. However, it is possible to evaluate the existence of micronic birch pollen antigens in the air. In the present study, the appearance of micronic birch pollen antigens and birch pollen did not completely coincide. However, the former appeared several days after a large dispersion of pollen.

This phenomenon coincides with our previous results obtained with Cry j 1.[5] The origin of micronic birch pollen antigens cannot be determined at this stage; however, we can speculate as follows. The localization of Bet v in birch pollen grains was recently clarified.[10–12] According to El-Ghazaly *et al*.,[10] Bet v is mainly found in the starch granules and, to a slight extent, in the exine and intine. Lol P 9, one of the Gramineae pollen antigens, is localized in starch granules.[15] Suphioglu *et al*.[16] found starch granules with Lol p 9 antigens released from rye grass pollen in the air after rain. We investiageted whether Bet v also appeared after rain (or wet conditions) using methods previously used with Lol p 9 antigens. During our observations, it rained after the maximum pollen count had been obtained. Following the rain, micronic Bet v appeared in the air. Therefore, it is possible that starch granules with Bet v will release themselves from pollen grains when they are wet in the air. In the case of Japanese cedar, Cry j 2 is also localized in starch granules;[17] we should maintain our vigilence regarding Cry j 2 and investigate whether epidemics of asthma caused by Japanese cedar pollen antigens are associated with rain. In the case of grass pollen, spots against Phl p 5 were present in high numbers, whereas the air contained almost no grass pollen grains.[4] However, a large amount of Bet v was not found after the birch pollen season. Therefore, Bet v originates from birch pollen itself and is not derived from other plant parts or other cross-reactive particles originating in various organisms.

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